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$\frac{1}{n} \sum_{i=1}^n x_i = \bar{x}$

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ATGAATTTTCAAACAATCGAGCTTGACACATGGTATAGAAAATCTTATTTTGACCATTA
CATGAAGGAAGCGAAATGTTCTTTCAGCATCACGGCAAACGTCAATGTGACAAATTTG
CTCGCCGTGCTCAAGAAAAAGAAGCTCAAGCTGTATCCGGCTTTTATTTATATCGTAT
CAAGGGTCATTTCATTCGCGCCCTGAGTTTAGAACACGTTTGATGACAAAGGAAGCT
GGGTTATTGGGAACAAATGCATCCGTGCTATGCGATTTTTCATCAGGACGACCAAAC
GTTTTCCGCCCTCTGGACGGAATACTCAGACGATTTTTCGCAGTTTTATCATCAATAT
CTTCTGGACGCCGAGCGCTTTGGAGACAAAAGGGGCCCTTTGGGCTAAGCCGGACAT
CCCGCCCAATACGTTTTTCAGTTTCTTCTATTCCATGGGTGCGCTTTTCAACATTCAATT
TAAACCTTGATAACAGCGAACACTTGCTGCCGATTATTACAAACGGGAAATACTTTTC
AGAAGGCAGGGAAACATTTTTGCCCGTTTCCTGCAAGTTCACCATGCAGTGTGTGAC
GGCTATCATGCCGGCGCTTTTATAA (配列番号 58)

MNFQTIELDTWYRKS YFDHYMKEAKCSFSITANVNVTNLLAVLK KKKKLKLYPAFIYIVSRVI
HSRPEFRTTFDDKGQLGYWEQMHPCYAIFHQDDQTFSALWTEYSDDFSQFYHQYLLDA
ERFGDKRGLWAKPDIPPNTFSVSSIPWVRFSTFNLNLDNSEHLLPIITNGKYFSEGRETFL
PVSCKFTMQCVTAIMPALL (配列番号 59)

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MKKAVIVENKGCATCSIGAACLVDGPIPDFEIA GATGLFGLWG (配列番号 2)

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MKISMQKADFWKKAISLLVFTMFFTLMMSETVFAAGLNKDQKRRAEQLTSIFENGTTIEIQ
YGYVERLDDGRGYTCGRAGFTTATGDALEVVEVYTKAVPNKCLKKYLPRLRAKEESD
DTSNLKGFASAWKSLANDKEFRAAQDKVNDHLYYQPAMKRSDNAGLKTALARAVMYDT
VIQHGDDGDDPSFYALIKRTNKKAGGSPKDGIDEKKWLNKFLDVRYDDLMPANHDTRD
EWRESVARVDVLRSLAKENNYNLNGPIHVRSEYGNFVIK (配列番号8)

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MKRNQKEWESVSKKGLMKPGGTSIVKAAGCMGCWASKSIAMTRVCALPHPAMRAI
(配列番号6)

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ATGAAAAACAAGCCGCTCGCGTTTCAGATATGGGTTGTCATATCCGGCATCCTGTTAG
 CGATATCGATTTTACTGCTTGTGTTATTTTCAAACACGCTGCGAGATTTTTTCACTAAT
 GAAACGTATACGACGATTGAAAATGAGCAGCATGTTCTGACAGAGTACCGCCTGCCA
 GGTTCGATTGAAAGGCGCTATTACAGCGAGGAAGCGACGGCGCCGACAACCTGTCCG
 CTCCGTACAGCACGTGCTCCTTCCTGAAAATGAAGAGGCTTCTTCAGACAAGGATTTA
 AGCATTCTGTCATCTTCATTTATCCACAAGGTGTACAAGCTGGCTGATAAGCAGGAAG
 CTA AAAAGAAACGTTACAGCGCCGACGTCAATGGAGAGAAAGTGTTTTTGTCAATTA
 AAAGGGACTTTCCGTCAATGGACAATCAGCGATGATGCTCTCTTACGCGCTTGATTCT
 TATCGGGACGATTTGGCCTATACCTTGTTCAAACAGCTTCTGTTTATTATAGCTGTCGT
 CATTTTATTAAGCTGGATTCCGGCTATTTGGCTTGCAAAGTATTTATCAAGGCCTCTTG
 TATCATTTGAAAAACACGTCAAACGGATTTCTGAACAGGATTGGGATGACCCAGTAAA
 AGTGGACCGGAAAGATGAAATCGGCAAATTGGGCCATACCATCGAAGAGATGCGCC
 AAAAGCTTG TGCAAAGGATGAAACAGAAAGAACTCTATTGCAAATATCTCTCATGA
 TTTAAAAACGCCGGTCATGGTCATCAGAGGCTATACACAATCAATTAAGACGGGATT
 TTTCTAAAGGAGACCTTGAAAACACTGTAGATGTTATTGAATGCGAAGCTCTTAAGC
 TGGAGAAAAAAATAAAGGATTTATTATATTTAACGAAGCTGGATTATTTAGCGAAGCAA
 AAAGTG CAGCACGACATGTT CAGTATTGTGGAAGTGACAGAAGAAGTCATCGAACGA
 TTGAAGTGGGCGCGGAAAGAACTATCGTGGGAAATTGATGTAGAAGAGGATATTTTG
 ATGCCGGGCGATCCGGAGCAATGGAACAACTCCTCGAAAACATTTTGGA AAATCAA
 ATCCGCTATGCTGAGACAAAAATAGAAATCAGCATGAAACAAGATGATCGAAATATCG
 TGATCACCATTAAAAATGACGGTCCGCATATTGAAGATGAGATGCTCTCCAGCCTCTA
 TGAGCCTTTTAATAAAGGGAAGAAAGGCGAATTCGGCATTGGTCTAAGCATCGTAAAA
 CGAATTTTA ACTCTTCATAAGGCATCTATCTCAATTGAAAATGACAAAACGGGTGTATC
 ATACCGCATAGCAGTGCCAAAA (配列番号 17)

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MKNKPLAFQI WVVISGILLAISILLVLFSNTLRDFFTNETYTTIENEQHVLT EYRLPGSIE
 RRY YSEEATAPTTVRSVQ HVLLPENEEASSDKDLSILS SSFIHKVYKLADKQEAKKR
 YSADVNGEKVFFVIKKGLSVNGQSAMMLS YALDSYRDDLAYTLFKQLLFIIAVVILLSWIPAI
 WLAKYLSRPLVSFEKHVKRISEQDWDDPVKVDRKDEIGKLGHTIEEMRQKLVQKDETER
 TLLQNISHDLKTPVMVIRGYTQSIKDGIFPKGDLENTVDVIECEALKLEKKIKDLLYLT KLDY
 LAKQKVQHDMFSIVEVTEEVIERLKWARKELSWEIVEEDILMPGDPEQWNKLL ENILENQI
 RYAETKIEISMKQDDRNIVITIKNDGPHIEDEMLSSLYEPFNKGKKGEFGIGLSIVKRIL TLHK
 ASISIENDKTGVSYRIAVPK (配列番号 18)

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MFKNNVILLNSPYHAHAHKEGFILKRGWTVLESKYDLLAQKYDCEEKVVTEIINLKAILNL
PKGTEHFVSDLHGEYQAFQHVLRNGSGRVKEKIRDIFSGVIYDREIDELAALVYPED
KLKLIKHDFAKEALNEWYKETIHRMIKLVSYCSSKYTRSKLRKALPAQFAYITEELLYK
TEQAGNKEQYYSEIIDQIIELGQADKLITGLAYSVQRLVVDHLHVVGDIYDRGPQPDRI
EELINYHSVDIQWGNHDLVWIGAYSGSKVCLANIIRICARYDNLDIEDVYGINLRPLL
LAKEYYDDNPAFRPKADENRPEDEIKQITKIHQAIAIMIQFKLESPIIKRRPNFNMEE
LEKIDYDKNEITLNGKTYQLENTCFATINPEQPDQLLEEEAEVIDKLLFSVQHSEKLGRH
MNFMMKKGSLYLKYNGLLIHGCIPVDENGNMETMMIEDKPYAGRELLDVFERFLREAF
AHPEETDDLATDMAWYLWTGEYSSLFGKRAMTTFFERYFIKEKETHKEKKNPYYYLREDE
ATCRNILAEFGLNPDHGHIIINGHTPVKEIEGEDPIKANGKMIVIDGGFSKAYQSTTGIAGYT
LLNSYGMQLVAHKHFNSKAEVLSTGTDVLTVKRLVDKELERKKVKETNVGEELLQEVAI
LESLERYRYMK (配列番号 20)

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MNSVDLTADLQALLTCPNVRHNL SAAQLTEKVL SRNEGILTSTGAVRATTGAYTRSPKD
KFIVEEESTKNKIDWGPVNQPISEEA FERLYTKVVSYLKERDEL FVFEGFAGADEKYRLPI
TVVNEFAWHNLFARQLFIRPEGNDKKTVEQPFTILSAPHFKADPKTDGTHSETFIIVSF
EKRTILIGGTEYAGEMKKSIFSIMN FLLPERDILSMHCSANVGEKGDVALFFGLSGTGKT
TLSADADRKLIGDDEHGWSDTG VFNIEGGCYAKCIHLSEEKEPQIFNAIRFGSVLENVV
DEDTREANYDDSFYTENTRAAYPIHMINNIVTPSMAGHPSAIVFLTADAFGVLPPISKLT
KEQVMYHFLSGYTSKLAGTERGVTSPETTFSTCFGSPFLPLPAHVYA EMLGKKIDEHGAD
VFLVNTGWTGGGYGTGERMKLSYTRAMV KAAIEGKLEDAEMITDDIFGLHIPAHVPGVPD
HILQPENTWTNKEEYKEKAVYLANEFKENFKKFAHTDAIAQAGGPLV (配列番号 28)

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MTVTYAHEPFTDFTEAKNKTAFGESLAFVNTQLGKHYPLVINGEKIETDRKIISINPANK
EEIIGYASTADQELAEKAMQAALQAFDSWKKQRPEHRANILFKAAAILRRRKHEFSSYL
KEAGKPWKEADADTAEIDFLEFYARQMLKLKEGAPVKSRAGEVNQYHYEALGVGIVISP
FNFPLAIMAGTAVAAIVTGNITLLKPADAAPVVAAKFVEVMEEAGLPNGVLNYIPGDGA
EIGDFLVEHPKTRFVSFTGSRVAVGCRIVERAAKVQPGQKWLKRVIAEMGGKDTVLVDK
DADLDAASSIVYSAFGYSGQKCSAGSRAVIHQDVYDEVVEKAVALTKLT TVGNPEDP
DTYMPVIHEASYNKVMKYIEIGKSEGLLAGGEGDDSKGYFIQPTIFADV DENARLMQ
EEIFGPVVAICKARDFDHMLEIANNTEYGLTGALLTKNRAHIERAREDFHVG
NL YFNRGCTGAIVGYQPFGGFNMSGTDSKAGGPDYLILHMQAKTTSEAF (配列番号 32)

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TAATACGATAAGAACAGCTTAGAAATACACAAGAGTGTGTATAAAGCAATTAGAATGA
GTTGAGTTAGAGAATAGGGTAGCAGAGAATGAGTTTAGTTGAGCTGAGACATTATGTT
TATTCTACCCAAAAGAAGTCTTTCTTTGGGTTTATTTGTTATATAGTATTTTATCCTCT
CATGCCATCTTCTCATTCTCCTTGCCATAAGGAGTGAGAGCAATGAATTTCCAATCAA
ACATTTCCGCATTTTTAGAGGACAGCTTGTCACCACACGATACCGATTGTGGAGAC
CTTCACAGTCGATACACTGACACCCATTCAAATGATAGAGAAGCTTGACAGGGAGATT
ACGTATCTTCTTGAAGCAAGGACGATACATCCACTTGGTCCAGATATTCGTTTATCG
GCCTGAATCCATTTCTACAATTAAGAAGAGCAGGGCCGTTTTTCGGCCGCTGATC
AGGACAGCAAATCTCTTACACAGGAAATGAACTAAAAGAAGTGCTGAACTGGATGAA
TACCACATACAAAATCAAACACCTGAGCTTGGCATTCTTTTTGTCGGCGGAGCTGTC
GGGTACTTAAGCTATGATATGATCCCGCTGATTGAGCCTTCTGTTCTTCGCATACCA
AAGAAACAGACATGGAAGGTGTATGCTGTTTGTTGCCGGACATTAATTGCGTATGA
TCATGAAACCAAAAACGTCCACTTTATCCAATATGCAAGGCTCACTGGAGAGGAAACA
AAAAACGAAAAAATGGATGTATTCATCAAATCATCTGGAGCTTCAAATCTCATTGA
AAAAATGATGGACCAAAAAAACATAAAAGAGCTGTTTCTTCTGCTGATTATACAAGA
CACCCAGCTTTGAGACAGTATCTTCTAATTATGAAAAATCGGCTTTTATGGCTGATGTA
GAAAAAATCAAAGCTATATAAAAGCAGGCGATATCTTCCAGGGTGTTTTATCACAAA
AATTTGAGGTGCCGATAAAAGCAGATGCTTTTGAGTTATACCGAGTGCTTAGGATCGT
CAATCCTTCGCCGTATATGTATTATATGAACTGCTAGACAGAGAAATAGTCGGCAGC
TCTCCGGAACGGTTAATACACGTTCAAGACGGGCACTTAGAAATCCATCCGATTGCC
GGTACGAGAAAACGCGGTGCAGACAAAGCTGAAGATGAGAGACTGAAGGTTGAGCT
CATGAAGGATGAAAAAGAAAAAGCGGAGCATTACATGCTCGTTGATCTTGCCCGAAA
CGATATCGGCAGAGTAGCAGAGTATGGTTCTGTTTCTGTGCCGGAGTTCACAAAAAT
TGTTTCTTTTACATGTCATGCACATTATCTCGGTGGTTACAGGCCGATTGAAAAA
GGGGTTCATCCTGTGATGCACTGATGTCTGCTTTCCCGGCGGGGACTTTAACAGGC
GCACCCAAAATCCGTGCCATGCAGCTTTTGCAAGAACTCGAGCCAACACCGAGAGAG
ACATACGGAGGGTGTATTGCCTACATTGGGTTTGACGGGAATATCGACTCTTGATTA
CGATTCGCACGATGAGTGTAAGAACGGTGTTGCATCGATACAGGCAGGTGCTGGC
ATTGTTGCTGATTCTGTTCCGGAAGCCGAATACGAAGAAAGCTGTAATAAAGCCGGT
GCGCTGCTGAAAACGATTCATATTGCAGAAGACATGTTTCATAGCAAGGAGGATAAA
GCTGATGAACAGATTTCTACAATTGTGCGTTGACGGAAAAACCTTACTGCCGGTGA
GGCTGAAACGCTGATGAATATGATGATGGCAGCGGAAATGACTCCTTCTGAAATGGG
GGGGATATTGTCAATTCTTGCTCATCGGGGGGAGACGCCAGAAGAGCTTGCGGGTT
TTGTGAAGGCAATGCGGGGCACACGCTCTTACAGTCGATGGACTTCCTGATATTGTTG
ATACATGCGGAACAGGGGGAGACGGTATTTCCACTTTTAAATATCTCAACGGCCTCGG
CAATTGTTGCCTCGGCAGCTGGTGCGAAAATCGCTAAGCATGGCAATCGCTCTGTCT
CTTCTAAAAGCGGAAGCGCTGATGTTTTAGAGGAGCTAGAGGTTTCTATTCAAACCAC
TCCCGAAAAGGTCAAAGCAGCATTGAAACAAACAACATGGGATTTCTTTTTGCGCCG
CTTTACCATTCTGTCTATGAAACATGTAGCAGGTAAGAAAAGAGCTAGGTTTCAGAA
CGGTATTTAATCTGCTTGCGCCGCTCAGCAATCCTTTACAGGCGAAGCGTCAGGTGA
TTGGGGTCTATTCTGTTGAAAAAGCTGGACTGATGGCAAGCGCACTGGAGACGTTTC
AGCCGAAGCACGTTATGTTTGTATCAAGCCGTGACGGTTTAGATGAGCTTTCAATTAC
AGCACCGACCGACGTGATTGAATTAAGGACGGAGAGCGCCGGGAGTATACCGTTT
CACCCGAAGATTTCGGTTTACAAATGGCAGACTTGAAGATTTACAGGTGCAGTCTCC
GAAAGAGAGCGCTTATCTCATTGAGAATATTTTTGAAAATAAAGCAGCAGTTCCGCT
TTATCTATTACGGCTTTTAAATGCGGGTGCTGCGATTTACACGGCGGGGAATTACCGCT
CACTGAAGGAAGGAACGGAGCTGGCGTTAGAGACGATTACAAGCGGAGGCGCTGCC
GCGCAGCTTGAACGACTAAAGCAGAAAGAGGAAGAGATCTATGCTTGAAAAATCAT
CAAACAAAAGAAAGAAGAAGTGAAAACACTGGTTCTGCCGGTAGAGCAGCCTTTCGA
GAAACGTTCATTTAAGGAGGCGCCGGCAAGCCCGAATCGGTTTATCGGGTTGATTGC
CGAAGTGAAGAAAGCATCGCCGTCAAAGGGCTTATTAAGAGGATTTTGTACCTGT
GCAGATTGCAAAAGACTATGAGGCTGCGAAGGCAGATGCGATTTCCGTTTAAACAGA
CACCCCGTTTTTTCAAGGGGAAAACAGCTATTTATCAGACGTAAAGCGTGCTGTTTCG
ATTCTGTACTTAGAAAAGATTTTATTATTGATTCTCTTCAAGTAGAGGAATCAAGAAG
AATCGGAGCGGATGCCATATTGTTAATCGGCGAGGTGCTTGATCCCTTACACCTTCAT
GAATTATATCTTGAAGCAGGTGAAAAGGGGATGGACGTGTTAGTGGAGGTTGATGAT
GCATCAACGCTAGAACAATATTGAAAGTGTTACACCCGACATTCTCGGCGTAAATA

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MNRFLQLCVDGKTLTAGEAETLMNMMMAAEMTPSEMGGILSILAHARGETPEELAGFVKA
MRAHALTVDGLPDIVDTCTGGDGISTFNISTASAIVASAAGAKIAKHGNRSVSSKSGSAD
VLEELEVSIQTTPEKVKSSIETNNMGFLFAPLYHSSMKHVAGTRKELGFRTVFNLLGPLSN
PLQAKRQVIGVYSVEKAGLMAALETTFQPKHVMFVSSRDGLDELSITAPTDVIELKDGER
REYTVSPEDFGFTNGRLEDLQVQSPKESAYLIQNIFENKSSSSALSITAFNAGAAIYTAGIT
ASLKEGTELALETITSGGAAAQLERLKKQKEEEIYA (配列番号 47)

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GTGATCACAAAGAGATTTTTCTATTTTTATCCAAAAGCGGCTTTCTCAATAAAATGGC
GAGGAACTGGGGAAAGTCGGGTAGCAGCGGGTAAAATTATCGGCGGGGAATGACTTTA
ACAGTTCAATCCCGACCATTGACAGCTTAACAGCCAAGGCTTGTCAGTTACTGTCGA
TCATTTAGGCGAGTTTGTGAACAGCGCCGAGGTCGCACGGGAGCGTACGGAAGAGT
GCATTCAAACCATTCGACCATCGCGGATCAGGAGCTGAACTCACACGTTTCTTTAA
AATGACGCTTTAGGTTTGGATATAGATATGGATTTGGTGTATGAAAATATGACAAAA
TCCTTCAGACGGCCGAGAAACATAAAATCATGGTCACCATTGACATGGAGGACGAAG
TCAGATGCCAGAAAACGCTTGATATTTTCAAAGATTTCAGAAAGAAATACGAGCATGT
GAGCACAGTGCTGCAAGCCTATCTGTACCGGACGGAAAAAGACATTGACGATTTGGA
TTCTTTAAACCCGTTCTCTTCGCCTTGTAAGGAGCTTATAAAGAATCAGAAAAAGTA
GCTTTCCCGGAGAAAAGCGATGTGATGAAAATTACAAAAAATCATCCGAAAGCAG
CTCTTAAACGGTCACTATACAGCGATTGCCACACATGACGACAAAATGATCGACTTTA
CAAAGCAGCTTGCCAAGGAACATGGCATTGCCAATGACAAGTTTGAATTTAGATGCT
GTACGGCATGCGGTCGCAAACCCAGCTCAGCCTCGTAAAAGAAGGTTATAACATGAG
AGTCTACCTGCCATACGGCGAGGATTGGTACGGCTACTTTATGAGACGCCTTGCAGA
ACGTCCGTCAAACATTGCATTTGCTTTCAAAGGAATGACAAAGAAG (配列番号 52)

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表1. プライマー

プライマー名	プライマー内に 設計された 制限酵素	プライマー配列	配列 番号
PBSX-UF	XbaI	CTACATTCTAGACGATTTGTTTGATCGATATGTGGAAGC	60
PBSX-UR	BamHI	GGCTGAGGATCCATTCTCAGCCCAGAAGAGAACCTA	61
PBSX-DF	BamHI	TCCCTCGGATCCGAAATAGGTTCTGCTTATTGTATTCCG	62
PBSX-DR	SacI	AGCGTTGAGCTCGCGCCATGCCATTATATTGGCTGCTG	63
7ア-シ [*] 1-	EcoRI	GTGACGGAATCCACGTGCGTCTTATATTGCTGAGCTT	64
7ア-シ [*] 1-	BamHI	CGTTTTGGATCCAAAAACACCCCTTTAGATAATCTTAT	65
7ア-シ [*] 1-	BamHI	ATCAAAGGATCCGCTATGCTCCAAATGTACACCTTTCCGT	66
7ア-シ [*] 1-	PstI	ATATTTCTGCAGGCTGATATAAATAATACTGTGTGTTCC	67
7ア-シ [*] 2-	SacI	CATCTTGAATCAAAGGGTACAAGCACAGAGACAGAG	68
7ア-シ [*] 2-	BamHI	TGACTTGGATCCGGTAAGTGGGCAGTTTGTGGGCAGT	69
7ア-シ [*] 2-	BamHI	TAGATAGGATCCTATTGAAAACCTGTTTAAGAAGAGGA	70
7ア-シ [*] 2-	PstI	CTGATTCTGCAGGAGTGTGTTTTGAAGGAAGCTTCATT	71
7ア-シ [*] 4-	KpnI	CTCCGCGGTACCGTCACGAATGCGCCTCTTATTCTAT	72
7ア-シ [*] 4-	BamHI	TCGCTGGGATCCTTGGCGCCGTGGAATCGATTTGTCC	73
7ア-シ [*] 4-	BamHI	GCAATGGGATCCTATATCAACGGTTATGAATTCACAA	74
7ア-シ [*] 4-	PstI	CCAGAAGTGCAGGAGCGAGGCGTCTCGCTGCCTGAAA	75
PPS-UF	SacI	GACAAGGAGCTCATGAAAAAAGCATAAAGCTTTATGTTGC	76
PPS-UR	BamHI	GACAAGGGATCCCGGCATGTCCGTTATTACTTAATTTTC	77
PPS-DF	BamHI	GACAAGGGATCCTGCCGCTTACCGGAAACGGA	78
PPS-DR	XbaI	GACAAGTCTAGATTATCGTTTGTGCAGTATTACTTG	79
SPβ-UF	SacI	ACTGATGAGCTCTGCCTAAACAGCAAACAGCAGAAC	80
SPβ-UR	BamHI	ACGAATGGATCCATCATAAAGCCGCAGCAGATTAATAT	81
SPβ-DF	BamHI	ACTGATGGATCCATCTTCGATAAATATGAAAGTGGC	82
SPβ-DR	XbaI	ACTGATTCTAGAGCCTTTTCTCTTGATGCAATTCCTC	83
PKS-UF	XbaI	GAGCCTCTAGAGCCCATTTGAATCATTTGTTT	84
PKS-UR	BamHI	GAGCCGGATCCTTAAGGATGTCGTTTTGTGTCT	85
PKS-DF	BamHI	GAGCCGGATCCATTCGGGGTTCTCAAAAAA	86
PKS-DR	SacI	GAGCCGAGCTCATGCAAAATGGAAAAATTGAT	87
λキ [*] -UF	XbaI	GAAGTTCTAGAGATTGTAATTACAAAAGGGGGTG	88
λキ [*] -UR	BamHI	GAAGTGGATCCTTTACCCGATCATAAAAGCCC	89
λキ [*] -DF	BamHI	TGAAAGGATCCATTTTTCATTGATTGTTAAGTC	90
λキ [*] -DR	SacI	GAAGTTAGAGCTCGGGGGGGCATAAATTTCCCG	91
Phleo-UF	BamHI	GCTTATGGATCCGATACAAGAGAGGTCTCTCG	92
Phleo-DR	BamHI	GCTTATGGATCCCTGTCATGGCGCATTAACG	93
Spec-UF	BamHI	ACTGATGGATCCATCGATTTTCGTTTCGTGAATACATG	94
Spec-DR	BamHI	ACTGATGGATCCCATATGCAAGGGTTTATTGTTTTTC	95
CssS-UF	XbaI	GCACGTTCTAGACCACCGTCCCCTGTGTTGTATCCAC	96
CssS-UR	BamHI	AGGAAGGGATCCAGAGCGAGGAAGATGTAGGATGATC	97
CssS-DF	BamHI	TGACAAGGATCCTGTATCATACCGCATAGCAGTGCC	98

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CssS-DR	SacI	TTCCGCGAGCTCGGCGAGAGCTTCAGACTCCGTCAGA	99
SBO-	XbaI	GAGCCTCTAGATCAGCGATTTGACGCGGCGC	100
SBO-	BamHI	TTATCTGGATCCCTGATGAGCAATGATGGTAAGATAGA	101
SBO-	BamHI	GGGTAA GGATCC CCCAAAAGGGCATAGTCATTCTACT	102
SBO-	Asp718	GAGATCGGTACC CTTTTGGGCCATATCGTGGATTTC	103
PhrC-UF	HindIII	GAGCC AAGCTT CATTGACAGCAACCAGGCAGATCTC	104
PhrC-DF	PstI	GCTTATAAGCTTGATACAAGAGAGGTCTCTCG	105
PhrC-UR	PstI	GCTTATAAGCTTCTGTCATGGCGCATTAAACG	106
PhrC-DR	SacI	GAGCCGAGCTC CATGCCGATGAAGTCATCGTCGAGC	107
PhrC-UF-	HindIII	CGTGAA AAGCTT TCGCGGGATGTATGAATTTGATAAG	108
PhrC-DR-	SacI	TGTAGGGGAGCTC GATGCGCCACAATGTCGGTACAACG	109

制限酵素認識部位を以下の通り指定する： XbaI は TCTAGA；BamHI は GGATCC；SacI は GAGCTC；Asp718 は GGTACC；PstI は CTGCAG 及び HindIII は AAGCTT。また、P^o7^oア-ジは“P7^oア-ジ”とする。

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表 2. 欠失構築体で使用する固有制限酵素ペア

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表 3. プライマー

プライマー名	プライマー内に 設計された 制限酵素	配列	配列 番号
DHB-UF	XbaI	CGAGAATCTAGAACAGGATGAATCATCTGTGGCGGG	110
DHB-UFfus-phleo	BamHI	CGACTGTCCAGCCGCTCGGCACATCGGATCCGCTTA CCGAAAGCCAGACTCAGCAA	111
DHB-URfus-phleo	BamHI	TTGCTGAGTCTGGCTTTCGGTAAGCGGATCCGATGTG CCGAGCGGCTGGACAGTCG	112
DHB-DFfus-phleo	BamHI	CGTTAATGCGCCATGACAGCCATGAGGATCCACAA GCCCCGACGCCTTGCCACAC	113
DHB-DRfus-phleo	BamHI	GTGTGGCAAGGCGTGC GGGCTTGTGGGATCCTCATG GCTGTCATGGCGCATTAAACG	114
DHB-DR	SacI	GACTTCGTCGACGAGTGC GGCAGGCCAGCATCACCA	115
DHB-UF-ネステイト	XbaI	GGCATATCTAGAGACATGAAGCGGGAAACAGATG	116
DHB-DR-ネステイト	SacI	GGTGC G GAGCTCGACAGTATCACAGCCAGCGCTG	117
YvfF-yveK-UF	XbaI	AAGCGTTCTAGACTGCGGATGCAGATCGATCTCGGG	118
YvfF-yveK-UF- phleo	BamHI	AACCTTCCGCTCACATGTGAGCAGGGGATCC GCTTACCGAAAGCCAGACTCAGCAA	119
YvfF-yveK-UR- phleo	BamHI	TTGCTGAGTCTGGCTTTCGGTAAGCGGATCC CCTGCTCACATGTGAGCGGAAGGTT	120
YvfF-yveK-DF- phleo	BamHI	CGTTAATGCGCCATGACAGCCATGAGGATCC GCCTTCAGCCTTCCCGCGGCTGGCT	121
YvfF-yveK-DR- phleo	BamHI	AGCCAGCCGCGGGAAGGCTGAAGGCGGATCC TCATGGCTGTCATGGCGCATTAAACG	122
YvfF-yveK-DR	PstI	CAAGCACTGCAGCCCACACTTCAGGCGGCTCAGGTC	123
YvfF-yveK-UF-	XbaI	GAGATATCTAGAATGGTATGAAGCGGAATTCCCG	124
YvfF-yveK-DR-	KpnI	ATAAACGGTACCCCCCTATAGATGCGAACGTTAGCCC	125
7° 7-UF	EcoRI	AAGGAGGAATTCCATCTTGAGGTATACAAACAGTCAT	126
7° 7-UF-	BamHI	TCTCCGAGAAAGACAGGCAGGATCGGGATCC	127
7° 7-UR-	BamHI	TTGCTGAGTCTGGCTTTCGGTAAGCGGATCC	128
7° 7-UR-	BamHI	TTGCTGAGTCTGGCTTTCGGTAAGCGGATCC	129
7° 7-UR-	Asp718	AAGGACGGTACCGGCTCATTACCCTCTTTTCAAGGGT	129
7° 7-UR-	BamHI	ACCAAAGCCGGAAGTCCCCGCGAGAGGATCC GCTTACCGAAAGCCAGACTCAGCAA	130
7° 7-UR-	BamHI	TTGCTGAGTCTGGCTTTCGGTAAGCGGATCC TCTCGCGGGGGAGTCCGGCTTTGGT	131
7° 7-UR-	BamHI	CGTTAATGCGCCATGACAGCCATGA GGATCCCATACGGGGTACACAATGTACCATA	132
7° 7-UR-	BamHI	TATGGTACATTGTGTACCCCGTATGGGATCC TCATGGCTGTCATGGCGCATTAAACG	133
7° 7-UR-	PstI	GTCAACCTGCAGAGCGGCCAGGTACAAGTTGGGGA	134
7° 7-UR-	SacI	GGATCAGAGCTCGCTTGTCTCTCTGGGAACAGCCGG	135
7° 7-UR-	PstI	TATATGCTGCAGGGCTCAGACGGTACCGGTTGTTCT	136

制限酵素認識部位は以下の通り指定される： XbaI は TCTAGA； BamHI は GGATCC；
SacI は GAGCTC； Asp718 は GGTACC； PstI は CTGCAG 及び HindIII は AAGCTT。

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表4. PCR融合*により欠失構築体を作成するために用いる追加プライマー

プライマー名	プライマー内に 設計された 制限酵素	配列	配列 番号
Slr-UF	XbaI	CTGAACCTCTAGACCTTCACCAGGCACAGAGGAGGTGA	137
Slr-Uffus	BamHI	GCCAATAAGTTCTCTTTAGAGAACAGGATCC GCTTACCGAAAGCCAGACTCAGCAA	138
Slr-Urfus	BamHI	TTGCTGAGTCTGGCTTTTCGGTAAGCGGATCCTTGTCTCT AAAGAGAACTTATTGGC	139
Slr-Dffus	BamHI	CGTTAATGCGCCATGACAGCCATGAGGATCC GGGCTAACGTTTCGCATCTATAGGGG	140
Slr-Drfus	BamHI	CCCCTATAGATGCGAACGTTAGCCC GGATCC TCATGGCTGTCATGGCGCATTAACG	141
Slr-DR	SacI	TGAGACGAGCTCGATGCATAGGCGACGGCAGGGCGCC	142
Slr-UF-ネステイト*	XbaI	CGAAATTCTAGATCCCGCGATTCCGCCCTTTGTGG	143
Slr-DR-ネステイト*	SacI	TTCCAAGAGCTCGCGGAATACCGGAAGCAGCCCC	144
YbcO-UF	XbaI	CAATTCTCTAGAGCGGTCGCGCGAGGTATAGGAGGGG	145
YbcO-UF	BamHI	GAAAAGAAACCAAAAGAATGGGAAGGATCC GCTTACCGAAAGCCAGACTCAGCAA	146
YbcO-UR	BamHI	TTGCTGAGTCTGGCTTTTCGGTAAGCGGATCC TTCCCATCTTTTGGTTTCTTTTC	147
YbcO-DF	BamHI	CGTTAATGCGCCATGACAGCCATGAGGATCC GCTATTTAACATTTGAGAATAGGGA	148
YbcO-DR	BamHI	TCCCTATTCTCAAATGTTAAATAGCGGATCC TCATGGCTGTCATGGCGCATTAACG	149
YbcO-DR	SacI	CAGGCGGAGCTCCCATTTATGACGTGCTTCCCTAAGC	150
Csn-UF	XbaI	TACGAATCTAGAGATCATTGCGGAAGTAGAAGTGGAA	151
Csn-UF	BamHI	TTTAGATTGAGTTCATCTGCAGCGGGGATCC GCTTACCGAAAGCCAGACTCAGCAA	152
Csn-UR	BamHI	TTGCTGAGTCTGGCTTTTCGGTAAGCGGATCC CCGCTGCAGATGAACTCAATCTAAA	153
Csn-DF	BamHI	CGTTAATGCGCCATGACAGCCATGAGGATCC GCCAATCAGCCTTAGCCCTCTCAC	154
Csn-DR	BamHI	GTGAGAGGGGCTAAGGCTGATTGGCGGATCC TCATGGCTGTCATGGCGCATTAACG	155
Csn-DR	Sall	ATACTCGTCGACATACGTTGAATTGCCGAGAAGCCGC	156
Csn-UF-	なし	CTGGAGTACCTGGATCTGGATCTCC	157
Csn-DR-	なし	GCTCGGCTTGTTTCAGCTCATTTCC	158
SigB-UF	SacI	CGGTTTGAGCTCGCGTCCTGATCTGCAGAAGCTCATT	159
SigB-UF	BamHI	CTAAAGATGAAGTCGATCGGCTCATGGATCC GCTTACCGAAAGCCAGACTCAGCAA	160
SigB-UR	BamHI	TTGCTGAGTCTGGCTTTTCGGTAAGCGGATCC ATGAGCCGATCGACTTCATCTTTAG	161

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SigB-DF	BamHI	CGTTAATGCGCCATGACAGCCATGAGGATCC GAAGATCCCTCGATGGAGTTAATGT	162
SigB-DR	BamHI	ACATTAACTCCATCGAGGGATCTTCGGATCC TCATGGCTGTCATGGCGCATTAAACG	163
SigB-DR	Sall	GCTTCGGTCGACTTTGCCGTCTGGATATGCGTCTCTCG	164
SigB-UF-	SacI	GTCAAAGAGCTCTATGACAGCCTCCTCAAATTGCAGG	165
SigB-DR-	Sall	TTCCATGTGACGCTGTGCAAACCGCCGGCAGCGCC	166
SpolISA-UF	EcoRI	ACATTCGAATTCAGCAGGTCAATCAGCTCGCTGACGC	167
SpolISA-UF	BamHI	CCAGCACTGCGCTCCCTCACCCGAAGGATCC GCTTACCGAAAGCCAGACTCAGCAA	168
SpolISA-UR	BamHI	TTGCTGAGTCTGGCTTTCGGTAAGCGGATCC TTCGGGTGAGGGAGCGCAGTGCTGG	169
SpolISA-DF	BamHI	CGTTAATGCGCCATGACAGCCATGAGGATCC TCGAGAGATCCGGATGGTTTTCTGT	170
SpolISA-DR	BamHI	CAGGAAAACCATCCGGATCTCTCGAGGATCC TCATGGCTGTCATGGCGCATTAAACG	171
SpolISA-DR	HindIII	AGTCAT AAGCTTTCTGGCGTTTGATTCATCAACGGG	172
SpolISA-UF-	なし	CAGCGCGACTTGTTAAGGGACAATA	173
SpolISA-DR-	なし	GGCTGCTGTGATGAACCTTTGTCGGA	174

*全ての欠失構築体は phleo^R マーカーを含む

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表 6. sigD 構築のためのプライマー

プライマー名	プライマー内に 設計された 制限酵素	プライマー配列	配列 番号
SigD UF	なし	ATATTGAAGTCGGCTGGATTGTGG	183
SigD UR	BglII	GCGGCAGATCTCGGCGCATTAAAGTCGTCA	184
SigD DF	EcoRI	GCGGCGAATTCTCTGCTGGAAAAAGTGATACA	185
SigD DR	なし	TTCGCTGGGATAACAACAT	186
Loxspc UF	BglII	GCGGCAGATCTTAAGCTGGATCCATAACTTCG	187
Loxspc DR	EcoRI	GCGGCGAATTCATATGGCGGCCGCATAACTTC	188
SigD UO	なし	CAATTTACGCGGGGTGGTG	189
SigD DO	なし	GAATAGGTTACGCAGTTGTTG	190
Spc UR	なし	CTCCTGATCCAAACATGTAAG	191
Spc DF	なし	AACCCCTTGCATATGTCTAG	192

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表7. **prpC** 欠失プライマー

プライマー名	プライマー内に設計された制限酵素	プライマー配列	配列番号
p95 DF	なし	GCGCCCTTGATCCTAAGTCAGATGAAAC	193
p96 UR	なし	CGGGTCCGATACTGACTGTAAGTTTGAC	194
p100 spc R	PfIMI	GTACCATAACCATGCCTTGTTAGGATGCATATGGCGGCCGC	195
p101 spc F	BstXI	CCTTGTCTTCCATCTTGCTGGAGCTGGATCCATAACTTCGTATAATG	196
p114 anal.	なし	GAGAGCAAGGACATGACATTGACGC	197
p115 anal.*	なし	GATCTTCACCCTCTTCAACTTGTAAG	198

*anal、分析 PCR プライマー

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表 8. PckA 欠失のために用いるプライマー

プライマー名	プライマー内に 設計された 制限酵素	プライマー配列	配列 番号
pckA UF	なし	TTTGCTTCCTCCTGCACAAGGCCTC	199
pckA-2URfus	なし	CGTTATTGTGTGTGCATTTCCATTGT	200
spc ffus	なし	CAATGGAAATGCACACACAATAACGTGACTGGCAA GAGA	201
pckA DFfus	なし	GTAATGGCCCTCTCGTATAAAAAAC	202
spc rfus	なし	GTTTTTTATACGAGAGGGCCATTACCAATTAGAAT GAATATTTCCC	203
pckA DR	なし	GACCAAAATGTTTCGATTGAGCATTCCT	204

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GGGGATCTCTGCAGTGAGATCTGGTAATGACTCTCTAGCTTGAGGCATCAAATAAAACGAAAG
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CGGTGTAGATGTTGATGAATTATTAAGAAAACAACAACAGATAGATATAAATTAATTAAAGAAA
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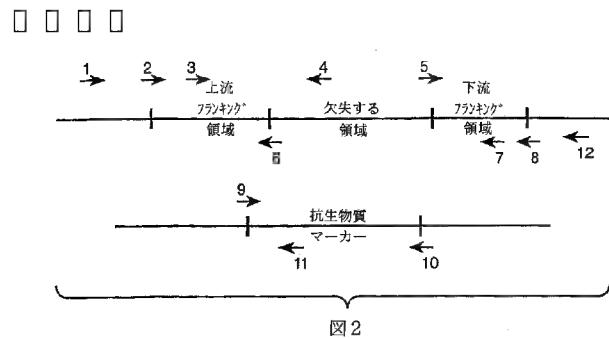
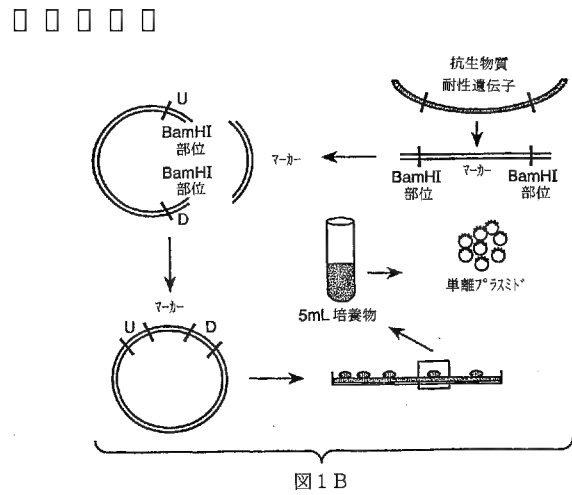
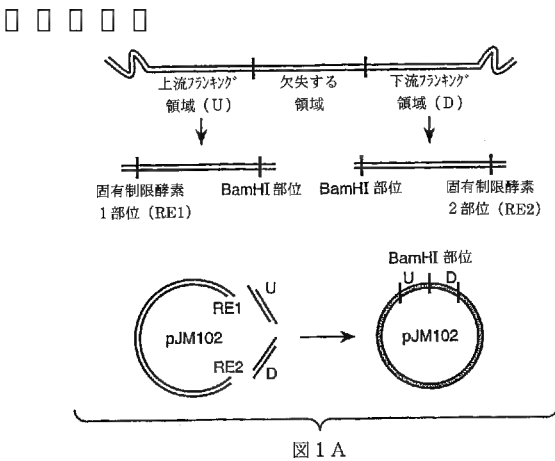
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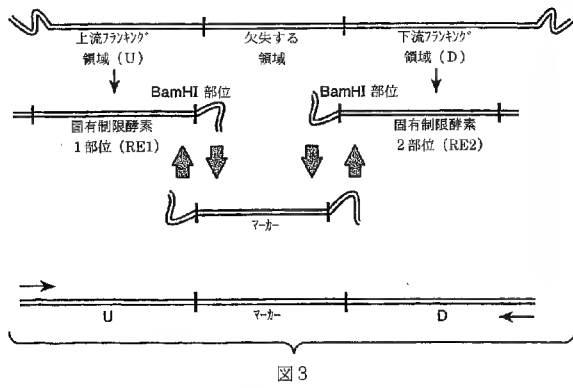


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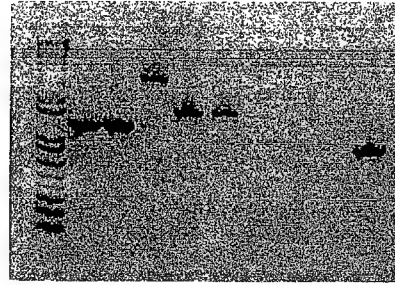


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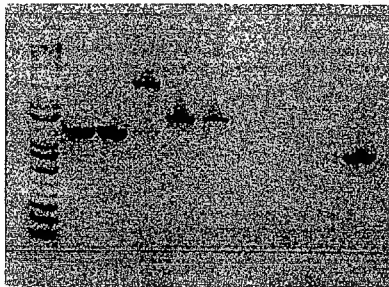


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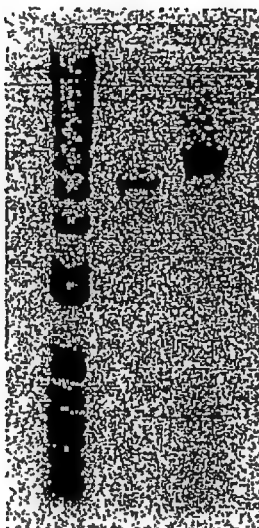


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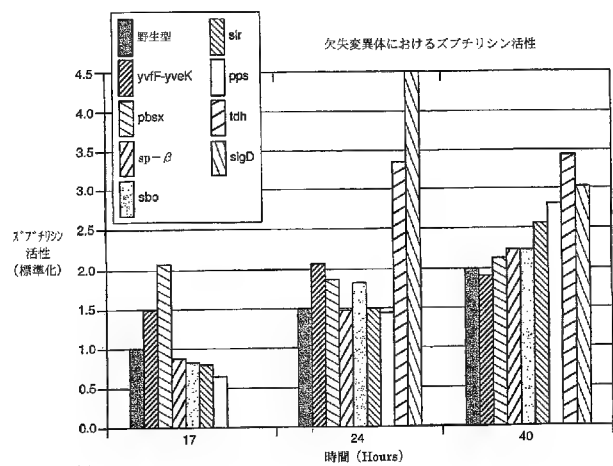


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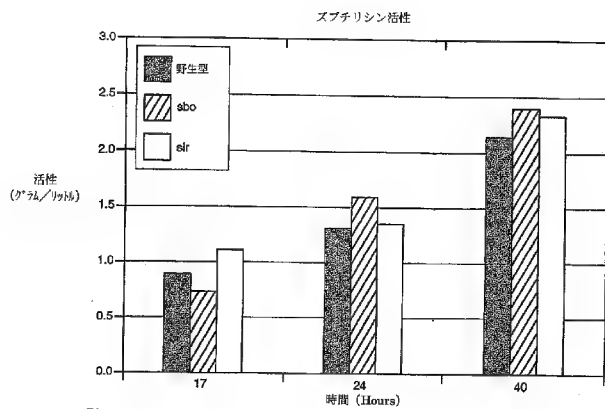


図 8

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INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/09585

A. CLASSIFICATION OF SUBJECT MATTER

IPC(7) : C12P 21/06; C12N 9/54, 1/20, 15/00, 15/74, 1/00

US CL : 435/252.31, 252.5, 221, 69.1, 440, 832, 471, 69.3

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)

U.S. : 435/252.31, 252.5, 221, 69.1, 440, 832, 471, 69.3

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practicable, search terms used)
Please See Continuation Sheet

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	US 5,387,521 A (FERRARI) 07 February 1995, see entire document.	1-13
Y	YANG et al. Alanine-scanning mutagenesis of Bacillus subtilis trp RNA-binding attenuation protein (TRAP) reveals residues involved in tryptophan binding and RNA binding. J. Mol. Biol. 1997, Vol. 270, pages 696-710, see entire document.	1-13
Y, P	US 6,528,255 B1 (ESTELL) 04 March 2003, see entire document.	1-13
Y	US 5,585,253 A (DOI et al.) 17 December 1996, see entire document.	1-13
Y	WO 99/03984 A2 (GENENCOR INTERNATIONAL, INC) 28 January 1999, see entire document.	1-13
A	YAKHNIN et al. Expression of the Bacillus subtilis trpEDCFBA operon is influenced by translational coupling and Rho termination factor. J. Bacteriology. October 2001, Vol. 183, No. 20, pages 5918-5926, see entire document.	1-13
A	EP 0 246 678 A1 (GENENTECH, INC) 25 November 1987, see entire document.	1-13

☒ Further documents are listed in the continuation of Box C.☐ See patent family annex.

* Special categories of cited documents:

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier application or patent published on or after the international filing date

"L" document which may throw doubt on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T"

later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X"

document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y"

document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art

"&"

document member of the same patent family

Date of the actual completion of the international search

29 July 2003 (29.07.2003)

Date of mailing of the international search report

22 AUG 2003

Name and mailing address of the ISA/US

Mail Stop PCT, Attn: ISA/US
Commissioner for Patents
P.O. Box 1450
Alexandria, Virginia 22313-1450

Facsimile No. (703)305-3230

Authorized officer

Nina M. Mumfield

Telephone No. 703-308-0196

Form PCT/ISA/210 (second sheet) (July 1998)

SUBSTITUTE SHEET (RULE 26)

INTERNATIONAL SEARCH REPORT

PCT/US03/09585

C. (Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT		
Category *	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 92/19721 A1 (GENENCOR INTERNATIONAL, INC) 12 November 1992, see entire document.	1-13
Y	US 5,310,675 A (ESTELL et al) 10 May 1994, see entire document.	1-13
Y	US 5,264,366 A (FERRARI et al) 23 November 1993, see entire document.	1-13
A,P	US 6,509,185 B1 (VALLE et al) 21 January 2003, see entire document.	1-13

INTERNATIONAL SEARCH REPORT

International application No.

PCT/US03/09585

Box I Observations where certain claims were found unsearchable (Continuation of Item 1 of first sheet)

This international report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. ☐ Claim Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:
2. ☐ Claim Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:
3. ☐ Claim Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of Item 2 of first sheet)This International Searching Authority found multiple inventions in this international application, as follows:
Please See Continuation Sheet

1. ☐ As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.
2. ☐ As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.
3. ☐ As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:
4. ☒ No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.: 1-13

Remark on Protest

☐
☐

The additional search fees were accompanied by the applicant's protest.

No protest accompanied the payment of additional search fees.

INTERNATIONAL SEARCH REPORT

PCT/US03/09585

BOX II. OBSERVATIONS WHERE UNITY OF INVENTION IS LACKING

Group I, claims 1-13, drawn to altered *Bacillus* strain and method for enhancing expression of protein.

Group II, claims 14-37, drawn to DNA construct.

Group III, claims 38-49, drawn to method for enhancing expression of a protease.

Group IV, claims 50-60, drawn to an altered *Bacillus* strain.

Group V, claims 61-64, drawn to protease producing *Bacillus* strain.

Group VI, claims 65-72, drawn to method for expression of a protein of interest.

Group VII, claims 73-80, drawn to method for obtaining a protein of interest from a *Bacillus* strain.

Group VIII, claims 81-83, drawn to method for enhancing expression of a protein of interest in *Bacillus*.

Group IX, claims 84-85, drawn to method for enhancing expression of a protein of interest in *Bacillus*.

Group X, claims 86-87, drawn to a nucleic acid sequence.

Group XI, claim 88, drawn to an amino acid sequence.

The inventions listed as Groups I-XI do not relate to a single general inventive concept under PCT Rule 13.1 because, under PCT Rule 13.2, they lack the same or corresponding special technical features for the following reasons: Groups I, II, IV, X and XI are drawn to different products each defining different technical features that lack unity of invention. The technical feature of Group I is not special in view of the teachings of Ferrari (5387521). Ferrari discloses an altered *Bacillus* strain that is capable of expressing proteins/enzymes. As such the various products and methods lack unity of invention because the technical feature of Group I is not "special" in that it does not define a novel contribution over the prior art. As such, the groups of inventions lack a corresponding "special" technical feature.

Continuation of B. FIELDS SEARCHED Item 3:

WEST, CABA, BIOSIS, MEDLINE, JAPIO, WPIDS, BIOTECHDS, LIFESCI, CAPLUS, AGRICOLA

search terms: inventor names, bacillus strain, alter?, mutant?, muta?, gene, heterologous protein, express?, inactivat? chromosomal gene, recited genes in claim 1

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(51) Int. Cl. ⁷

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